THE UTILIZATION OF SOME COORDINATION COMPOUNDS OF V(IV) AND CO(III) AS REGULATORS OF THE CONTENT OF BIOACTIVE SUBSTANCES WITH ANTIOXIDANT PROPERTIES AT *Spirulina platensis*

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Abstract. Investigation of non-traditional sources of bioactive substances is one of the current directions of biotechnology development in many countries worldwide. This paper reports on the study of possibility of utilization of some coordination compounds of V(IV) and Co(III) as regulators of the content of bioactive substances with antioxidant properties: superoxiddismutase (SOD), phycobiliproteins, carotenoids and lipids in the biomass of cyanobacterium *Spirulina platensis*. Natural antioxidants possess a broad spectrum of biological activity due to the ability to neutralize the negative effects of free radicals in living organisms. The obtained experimental results demonstrate that utilization of some coordination compounds of V(IV) at the spirulina cultivation contribute to the accumulation of β-carotene and lipids in spirulina biomass, as well as metallocomplexes of Co(III) have the beneficial effect on the content of phycobiliproteins, lipids and activity of SOD. The present study reveals that obtained biomass of cyanobacterium *Spirulina platensis* with high content of bioactive substances with antioxidant properties can be used for the elaboration of medical remedies for prophylaxis and treatment of diseases, caused by the negative effect of oxidative stress on live organisms and, also, for the elaboration of cosmetic preparations for the prevention of premature skin aging, protection against solar radiation and treatment of skin diseases.

Keywords*:* cyanobacterium *Spirulina platensis,* antioxidants, biologically active substances, coordination compounds of V(IV) and Co(III)

INTRODUCTION

Investigation of non-traditional sources of bioactive substances is one of the current directions of biotechnology development in many countries worldwide. Cyanobacterium *Spirulina platensis* is widely explored and used in recent decades as a source of valuable biologically active substances [1, 2, 32.]. An important role in neutralizing the harmful effects of oxidative stress have the enzime superoxiddismutase (SOD), that it is present, also, in the biomass of cyanobacterium *Spirulina platensis* [29, 35]. SOD is an important regulator of cell oxidation processes, acting for the conversion of O_2^- radicals with formation of hydrogen peroxide and triplet oxygen.

Summarize the most powerful antioxidants in spirulina biomass can be mentioned water-soluble pigments – phycobiliproteins, possessing anti-inflammatory, immunostimulatory, neuroprotective, hematopoietic properties [11, 16, 30]. According to the literature data, phycocyanin is an efficient scavenger of oxygen free radicals, reacts with other oxidants of pathological relevance such as HOCl and $ONOO^-$ and, also, inhibits the processes of lipid peroxidation, mediated by ROS (reactive oxygen species) [7-9].

Another group of pigments - carotenoids are widely used as natural dyes and antioxidants [3]. Carotenoids possess immunostimulatory properties and inhibit the growth of cancer cells that present the perspective of their use in prophylaxis and complex treatment of cancer [2]. Due to the presence of conjugated double bonds, carotenoids bind singlet oxygen and inhibit the formation of free radicals [21, 24].

Cyanobacterium *Spirulina platensis* presents, also, a source of polyunsaturated fatty acids which have a cardioprotective role, possess immunostimulatory, antiviral, antibacterial properties [6,17,18]. Previously, it was established that coordination compounds of Zn(II) with aminoacids and halogenoacetates can be used as effective regulators of lipids accumulation process by cyanobacterium *Spirulina platensis* [31].

Researches have demonstrated the possibility of obtaining of spirulina biomass with high content of zinc, iron, chromium, germanium, selenium, iodine and other bioactive substances at the cultivation of spirulina in the presence of coordination or inorganic compounds of these elements by the controlled synthesis [32]. It was established the stimulatory effect of coordination compounds of Mn (II) and Zn (II) on the activity of SOD and content of phycobiliproteins in spirulina biomass [15]. The differential response of superoxiddismutase (SOD), nitrogenase, growth and physiological processes in the presence of four different metals ions at varying concentrations were investigated in the heterocystous cyanobacterium *Anabaena variabilis* Kütz. Growth and enzyme activities were influenced by the metal ions. SOD activity was optimum at 100 mcM concentration of $FeSO_4$. In the presence of $ZnSO_4$ SOD activity increased in direct proportion with metal concentration [28].

The influence of vanadium compounds on photosynthesis in cyanobacteria hasn't been yet studied completely. Vanadium is suggested to act as a redox catalyst in the electron transport from PS II to PS I. Despite of the importance of vanadium as human micro-nutrient, it is yet to be unequivocally accepted by biologists and biomedical scientists, because of its toxicity Pharmacological uses of vanadium include lowering of cholesterol, triglycerides and glucose

levels [4, 19, 26]. Vanadium also possesses anti-carcinogenic and anti-diabetic properties [36]. The chemoprotective and anti-cancer effect of vanadium, a dietary micronutrient, against chemically induced hepatocarcinogenesis in rats was demonstrated [10] and, also, inhibition of growth of colon and lung tumors in human organism was established [20, 27].

Another trace element - cobalt has important biological significance, ions of which are actively involved in the reactions of oxidation and reduction, have a positive influence on the processes of cell respiration and metabolism, as well as on the biosynthesis of phycobiliproteins and nucleic acids [23, 37]. Thus, it can be assumed that synthesis of bioactive substances in spirulina biomass may be increased by the cultivation of this cyanobacteria in the presence of some compounds of Co (III).

The **aim** of this investigation presents a study of possibility of utilization of some coordination compounds of V(IV) and Co(III) as regulators of the content of bioactive substances with antioxidant properties (superoxide dismutase, phycobiliproteins, carotenoids, lipids) in the biomass of cyanobacterium *Spirulina platensis*.

MATERIALS AND METHODS

Object of study is a strain of cyanobacterium *Spirulina platensis CNM-CB-02*, stored at the National Collection of Nonpathogenic Microorganisms of the Institute of Microbiology and Biotechnology, Academy of Sciences of Moldova.

Cultivation of cyanobacterium *Spirulina platensis* was carried out on the nutritive medium *SP - 1* (Table 1) [33] with the certain ratio of macro-and micronutrients for normal growth and development of culture with the following composition:

Table 1. The composition of nutritive medium *SP-1* [33].

Reagents	at 1000 ml of nutritive medium
$NaHCO_3$	16.8g
K_2HPO_4	0.1g
KNO_3	3.75g
NaCl	1.0g
K_2SO_4	3.75g
$CaCl_2$	0.04g
$MgSO_4 \cdot 7H_2O$	0.70g
sol. Fe-EDTA	1.0 ml
sol. microelements	1.0 ml

Solution of microelements, mg/l: H_3BO_3 – 2.86; $MnCl_2 \cdot 4H_2O$ – 1.81; $ZnSO_4 \cdot 7H_2O$ – 0.22; MoO_3 – 0.015. Cultivation was carried out in Erlenmeyer flasks with a volume of a suspension of spirulina in the medium of cultivation 100 ml, for 144 hours at 30^0C, the intensity of illumination 49.5-66.0 μmol photons/m^2/s.

As stimulators of growth processes of cyanobacterium *Spirulina platensis* and accumulation of bioactive substances with antioxidant properties in the biomass coordination compounds of V(IV) and Co(II) with different ligands of organic nature:

L1 - $[(VO)_2(2PyTCH)]SO_4 \cdot 4H_2O$;
L2 - $[(VO)_2(2PyCH)]SO_4 \cdot 4H_2O$;
L3 - $[(VO)_2(2PyFx)]SO_4 \cdot 4H_2O$;
L4 - $[Co(L\text{-}H)En] \cdot 3H_2O$;
L5 - $Na[Co(DH)_2(NO_2)_2]$ have been used.

Productivity of spirulina was determined according to photocolorimetrical method [34].

The determination of activity of superoxide dismutase in the spirulina biomass was carried out according to the method proposed by Bulimaga [12]. The determination of activity of superoxiddismutase (SOD) in obtained extracts from spirulina biomass (by the utilization of 0.1 M Na - phosphate buffer pH 7.8 - 8.0 (+10 mM EDTA) is based on the ability of superoxide dismutase to inhibit the reduction of nitro-blue tetrazolium in the presence of TEMED and riboflavin . One unit is defined as that amount of enzyme causing half the maximum inhibition of NBT reduction.

The content of phycobiliproteins was determined according to spectrophotometrical method, elaborated by Bousiba and Richmond [11] in modification of [38]. Phycobiliprotein − phycocyanin (PC) and allophycocyanin (AP) concentrations were calculated from spectrophotometric method at 620 and 650 nm using equations. (1), (2).

PC (mg/mL)=(OD_{620nm} - 0.7x OD_{650nm})/7.38 (1)

AP (mg/mL) =(OD_{650nm} - 0.19 x OD_{620nm})/5.65 (2)

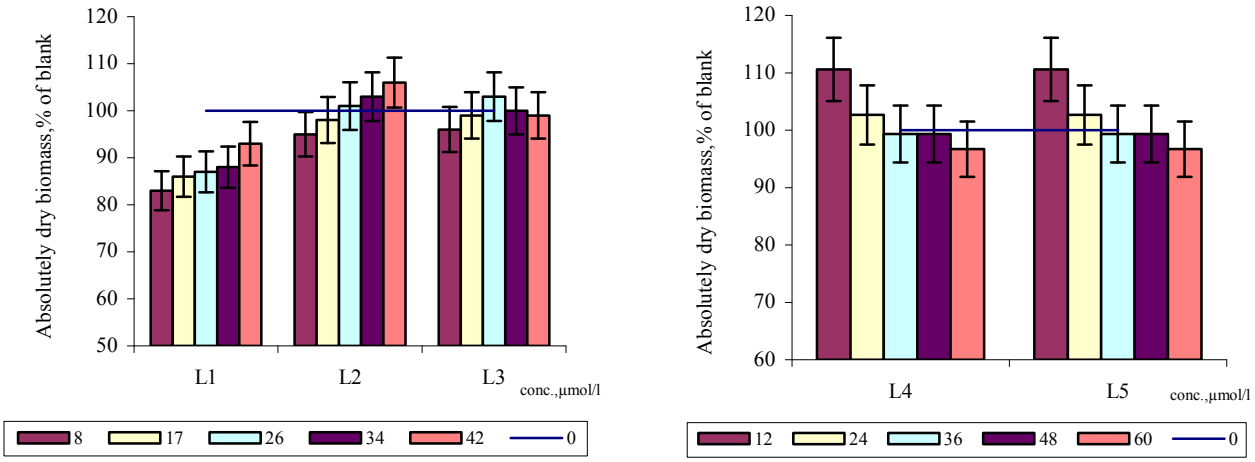
The carotenoids content in the biomass of spirulina was measured according to spectrophotometrical method [13]. Determination of the content of beta carotene was effectuated with the use of methanol and heptane as a solvent.

Determination of lipid contents was effectuated by spectrophotometrical method [34]. Principle of the method is based on the determination of acid hydrolysis products of lipids by the vanillin solution (10 mmol/l) in phosphoric acid. Staining intensity is directly proportional to the total lipid contents. Extinction is measured at 560 nm.

Regression and statistical analysis of data obtained in three series was carried out by the methods proposed by Maximov [40] and Dospehov [39].

RESULTS

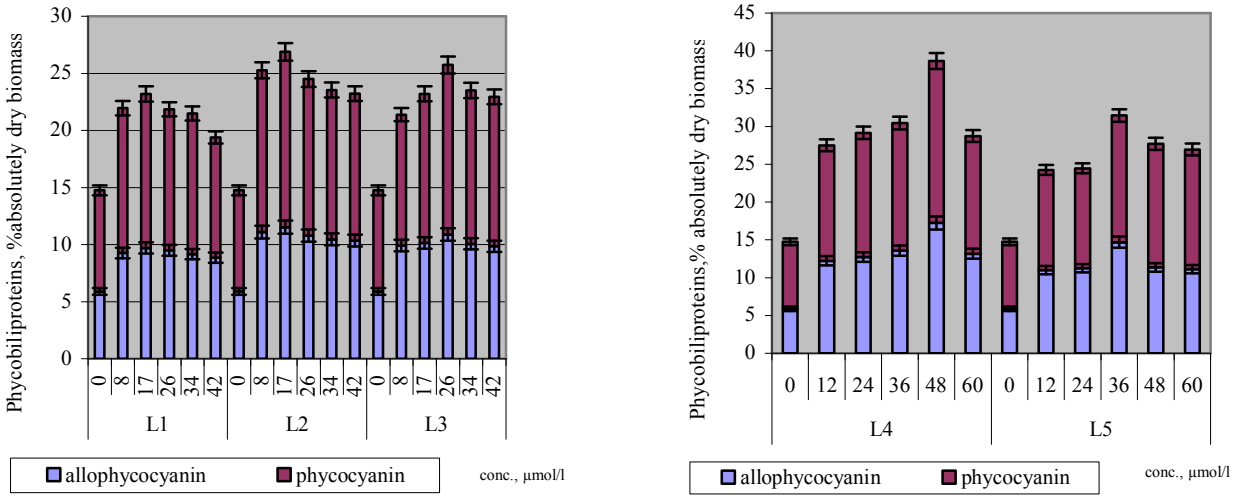
The high degree of biological activity of the cyanobacteria *Spirulina platensis* is caused mainly by the presence of antioxidants in the composition of its biomass. Results of investigation of influence of coordination compounds of some transition metals on productivity of spirulina and content of bioactive compounds with antioxidant properties in the biomass are presented below (Fig. 1-6).



a. **b.**

Figure 1. Productivity of spirulina at 7th day at the cultivation in the presence of some coordinative compounds of **a)** vanadium and **b)** cobalt. Data are means ± SD of three experiments (n=3). Bars with different letters differ significantly (P<0.05).

It was established a weak inhibitory effect of the two studied compounds: $[(VO)_2(2PyTCH)]SO_4 \cdot 4H_2O$ in concentrations within 8 to 42 µmol/l and $Na[Co(DH)_2(NO_2)_2]$ (24-60 µmol/l) on the productivity of spirulina. In the case of utilization of the other compounds, productivity values are within the reference sample, except for compounds $[Co(L\text{-}H)En] \cdot 3H_2O$ and $Na[Co(DH)_2(NO_2)_2]$, which contribute to increase of productivity by 11-18% compared to the reference sample in the concentration of 12 µmol/l (Fig. 1).

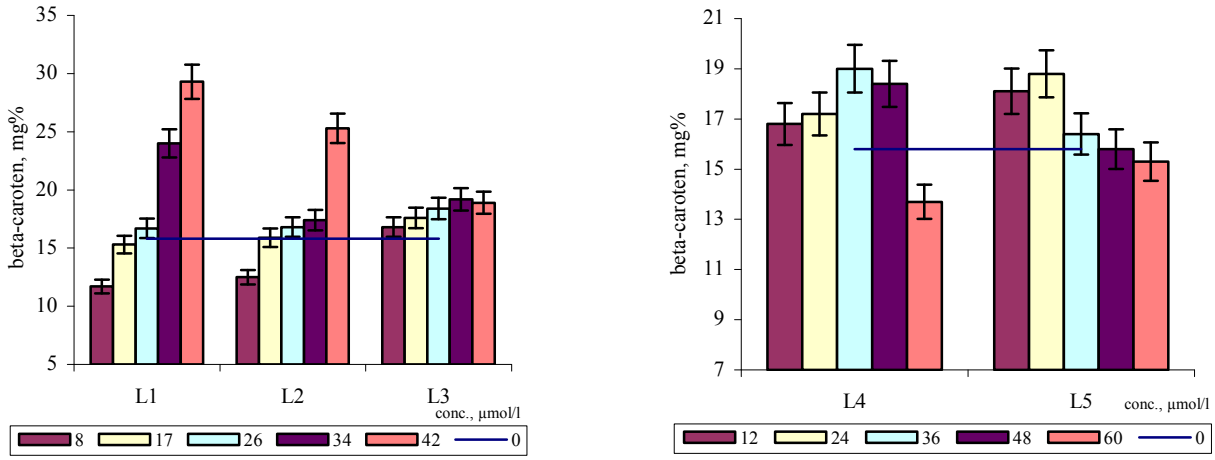


a. **b.**

Figure 2. Content of phycobiliproteins in the biomass of spirulina cultivated in the presence of some coordinative compounds of **a)** vanadium and **b)** cobalt. Data are means ± SD of three experiments (n=3). Bars with different letters differ significantly (P<0.05).

The analysis of obtained results has demonstrated that phycobiliproteins content is increased by 45-74% compared to the reference sample in the case of utilization of compounds of V(IV) (Fig. 2). The administration of the coordinative compound of Co (III) - $[Co(L\text{-}H)En] \cdot 3H_2O$ in the concentration of 48 µmol/l has recorded a maximum increase of phycobiliproteins contents (by 160% compared to the reference sample). The beneficial effect is caused possibly by the fact that coordination compounds of Co (III) causes a stimulation of electron transport in photosynthetic apparatus of the cell, therefore cells possess a greater fluidity of the thylakoid membrane, and also there is an enhancement of heme oxygenase synthesis, which causes the increase of phycobiliproteins content in the biomass [5]. Ligands of coordination compounds have a great role in regulation of biosynthetic activity of cyanobacteria. Probably, acetate, one of the ligands of the metalocomplex $[Co(L\text{-}H)En] \cdot 3H_2O$ may be included in the structure of precursors of phycobiliproteins, such as protoporphyrin, uroporphyrinogen and thereby contribute to enhancement the processes of biosynthesis of tetrapyrroles [22].

The significant stimulatory effect was established in the case of study of influence of some of complex compounds of V(IV) and Co (III) on the content of β - carotene in spirulina biomass (Fig. 3).

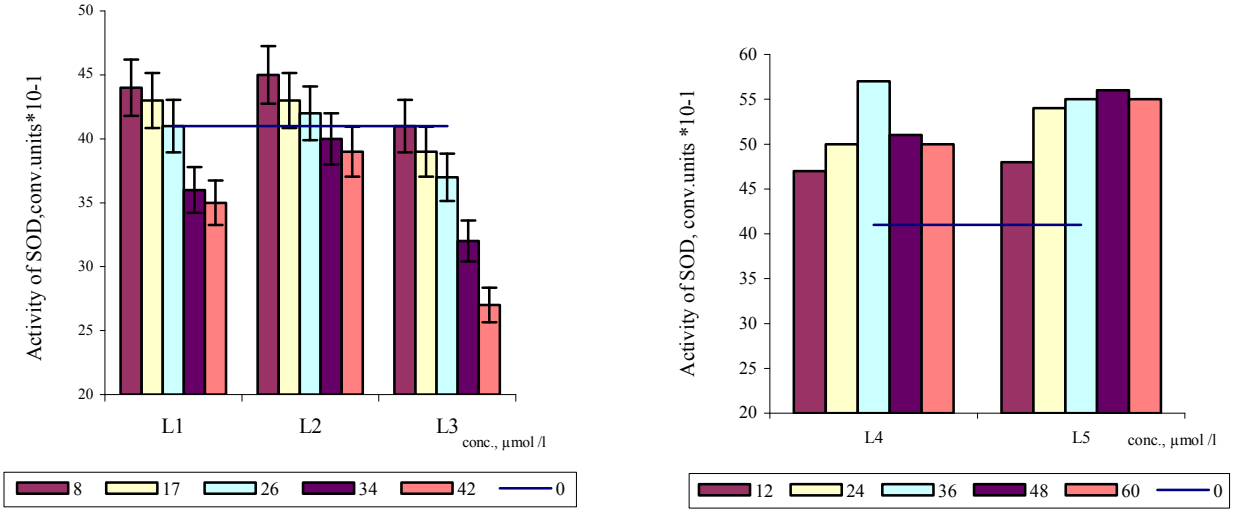


a. **b.**

Figure. 3. Content of β – carotene in the biomass of spirulina cultivated in the presence of some coordinative compounds of **a)** vanadium and **b)** cobalt. Data are means ± SD of three experiments (n=3). Bars with different letters differ significantly (P<0.05).

Beta-carotene contents in the biomass of spirulina cultivated in the presence of complexes $[(VO)_2(2PyTCH)]SO_4 \cdot 4H_2O$ and $[(VO)_2(2PyCH)]SO_4 \cdot 4H_2O$ reaches the values of 29 and 25mg%, respectively, at optimal concentration of compounds of 42 µmol /l.

The determination of activity of superoxiddismutase (SOD) in obtained extracts from spirulina biomass (by the utilization of 0.1 M Na - phosphate buffer pH 7.8 - 8.0 (+10 mM EDTA)) allowed to establish a positive effect of coordination compounds of Co (III) on the activity of this enzyme.



a. **b.**

Figure 4. The influence of some coordinative compounds on the activity of SOD in the biomass of spirulina **a)** vanadium and **b)** cobalt. Data are means ± SD of three experiments (n=3). Bars with different letters differ significantly (P<0.05).

Maximum increase of superoxiddismutase activity in the biomass of spirulina (by 38% compared to the reference sample) was established in the case of utilization of the compound $[Co(L\text{-}H)En]\cdot 3H_2O$ in the concentration of 36 µmol /l.

The significant increase of SOD activity (by 32-36% compared to the reference sample) is registered in the case of administration of the compound $Na[Co(DH)_2(NO_2)_2]$ in the concentration range of 36-60 µmol /l

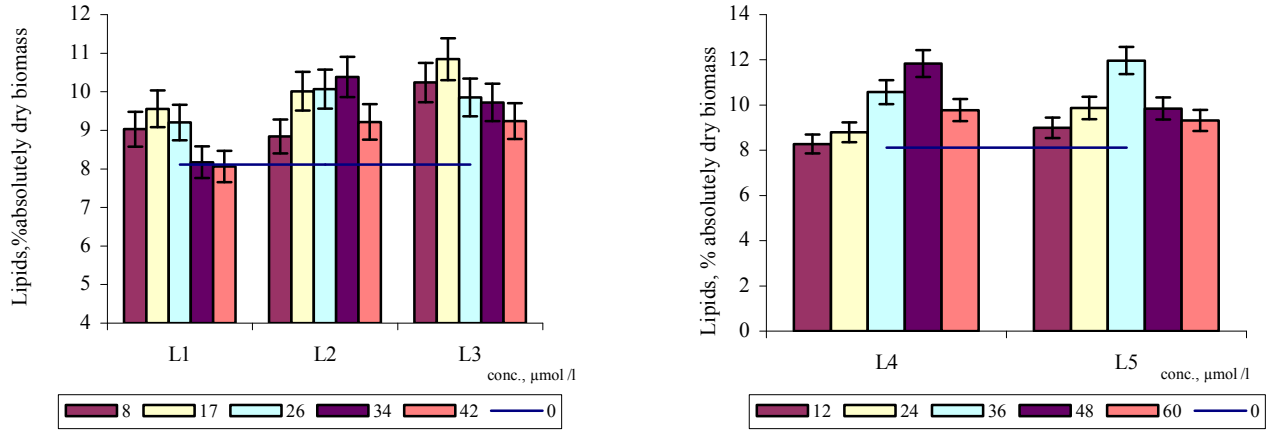
It was demonstrated that all tested compounds contribute to increased synthesis of lipids in biomass with a maximum of about 45-47% increase of their content compared to the reference sample in the case of utilization of coordination compounds of Co(III) - $[Co(L\text{-}H)En]\cdot 3H_2O$ and $Na[Co(DH)_2(NO_2)_2]$ in the optimal concentration of 48 and 36 µmol /l, respectively (Fig. 5).

Thus, can be indicated that the stimulatory effect of coordination compounds of V(IV) and Co(III) on the contents of bioactive substances with antioxidant properties is obvious and can be explained by the possible implications of these metals and ligands in metabolic processes cells.

DISCUSSIONS

The obtained experimental results reveal that utilization of coordination compounds of V(IV) at the

spirulina cultivation contributes to the accumulation of β - carotene and lipids in spirulina biomass, as well as metallocomplexes of Co(III) have the beneficial effect on the content of phycobiliproteins, lipids and activity of SOD. So, the administration of studied coordination compounds of V(IV) to the medium of cultivation of cyanobacterium *Spirulina platensis* provides the maximum increase of β - carotene contents with 60% compared to the reference sample in the case of utilization of compound $[(VO)_2(2PyTCH)]SO_4 \cdot 4H_2O$ in the optimal concentration of 42 µmol/l**.** In another study similar results were observed, where some vanadium compounds (vanadate, vanadyl citrate) have beneficial effect on the photosynthesis in *Chlorella fusca* and algal chloroplasts both vanadate and vanadyl citrate enhance photosynthetic O_2 production in intact cells [25].



a. **b.**

Figure 5. Content of lipids in the biomass of spirulina cultivated in the presence of some coordinative compounds of **a)** vanadium and **b)** cobalt. Data are means ± SD of three experiments (n=3). Bars with different letters differ significantly (P<0.05).

In the present investigation the maximal increase of phycobiliproteins content (160% compared to the reference sample) in the biomass of spirulina by the directed synthesis with the supplementation of metallocomplex of Co(III) - $[Co(L\text{-}H)En]\cdot 3H_2O$ in the optimal concentration of 48 µmol/l was reached. The data obtained is confirmed by other researchers, according to which some cobalt compounds are able to stimulate the processes of electron transport of PS II and phycobiliprotein synthesis in the biomass of cyanobacteria [5, 37].

According to the results of some researchers on the effect of inorganic forms of transition metals - sulfate of Zn(II) on SOD activity of spirulina the data are contradictory. Thus, the utilization of different concentrations of Zn^{2+} in the form of sulphate had inhibitory effect on the activity of antioxidant enzymes: superoxiddismutase and catalase in spirulina biomass collected at the end of exponential phase [41]. In another study, the authors revealed that the metal ions(Mn^{2+} and Zn^{2+}) in the form of sulphate play an important role in regulating of the SOD activity [28]. The utilization of coordinative compounds of zinc and manganese contributes, also, to the significant increase of SOD activity in the biomass of cyanobacteria *Spirulina platensis* [15]. In the present investigation an increasing of activity of superoxiddismutase in biomass of spirulina (by 38% compared to the reference sample) in the case of utilization of coordinative compound of Co(III) - $[Co(L\text{-}H)En]\cdot 3H_2O$ in optimal concentration of 36 µmol/l has been established.

In this research, for the first time, it was demonstrated the possibility of lipids directed synthesis in the biomass of spirulina by the coordinative compounds of V(IV) and Co(III). So, in the case of utilization of coordination compounds of Co(III) - $Na[Co(DH)_2(NO_2)_2]$ in the optimal concentration of 36 µmol/l an increase of lipid contents in the biomass of spirulina by 47% compared to the reference sample it was observed. Similar data was obtained at the utilization of the coordinative compound tribromoacetate of zinc $[Zn(CBr_3COO)_2\ 4H_2O]$ [31].

Probably, the assimilation of coordination compounds of transition metals with ligands of organic nature takes place due to the specific mechanism of transport of the metal-ligand complexes that lead to the increasing of metal concentration in the cell , it becomes possible to use higher concentrations of metal, causing oxidative stress in cells that contributes to the enhacement of synthesis of bioactive substances with antioxidants properties in the biomass of the cyanobacteria *Spirulina platensis.* Establishing of mechanism of action V (IV) and Co (III) coordination compounds includes the study of dissociation constant, mechanism of transport of metal-ligand complexes, processes of electron transport of PS II and phycobiliprotein synthesis and requires further research.

In conclusion, the present study reveals that obtained biomass of cyanobacterium *Spirulina platensis* with high content of bioactive substances with antioxidant properties (superoxiddismutase, phycobiliproteins, carotenoids, lipids) can be used for the elaboration of medical remedies for prophylaxis and treatment of diseases, caused by the negative effect of oxidative stress on live organisms and, also, for the elaboration of cosmetic preparations for the prevention

of premature skin aging, protection against solar radiation and treatment of skin diseases.

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Received: 10 December 2010
Accepted: 29 April 2011
Published Online: 4 May 2011
Analele Universităţii din Oradea – Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433